AMENDMENTS TO THE SPECIFICATION:

Please insert the following paragraph at page 1, line 1:

RELATED APPLICATIONS

The present application is a continuation of U.S. Application Serial No. 09/532,001, filed March 21, 2000, now U.S. Patent No. 6,946,246, which is a divisional of U.S. Application No. 09/056,363, filed April 7, 1998, now U.S. Patent No. 6,730,498, which claims the benefit of provisional U.S. Application Serial No. 60/043,205, filed April 8, 1997.

Please delete the amended paragraph beginning on page 1, line 13 with the heading "RELATED APPLICATIONS".

Please replace the paragraph beginning at page 8, line 22 with the following amended paragraph:

The present invention is directed to a method of producing a functional protein, comprising the steps of: isolating mammalian cells; placing said cells into a rotating wall vessel containing a cell culture comprising culture media and culture matrix; producing three-dimensional cell aggregates under simulated microgravity conditions; and detecting expression of the functional protein in the cell culture. Generally, simulated microgravity conditions comprise a balance between gravity and oppositely directed physical forces. Representative examples of such physical forces include sedimentational shear stress, centrifugal forces, viscosity and Coriolis ~~coriolus~~ forces.

Please replace the paragraph beginning at page 9, line 9 with the following amended paragraph:

Generally, any mammalian cell could be used in the methods of the present invention. Representative examples of mammalian cells include renal cortical cells, renal fibroblast cells, hepatocytes, pancreatic islets, renal interstitial cells, parathyroid cells,

thyroid cells, pituitary cells, ovarian cells and testicular cells. Generally, the cell is selected from the group consisting of epithelial cell and endothelial cell. Preferably, the cell contains shear stress response elements. Representative examples of shear stress response elements include GAGACC and GGTCTC.

Please replace the paragraph beginning at page 10, line 15 with the following amended paragraph:

As used herein, rotating wall vessels[[÷]] refers to a ~~cylidrical~~ cylindrical horizontal rotating culture vessel with a coaxial oxygenator.

Please replace the paragraph beginning at page 10, line 17 with the following amended paragraph:

As used herein, shear stress response element[[÷]] refers to a sequence of a family of genes in the cell nucleus which binds one or more transcription factors in response to shear stress on the cell. A representative example of a shear stress response element is GAGACC or its complementary sequence GGTCTC.

Please replace the paragraph beginning at page 10, line 21 with the following amended paragraph:

As used herein, shear stress conditions[[÷]] refers to flow of liquid, or current of liquid over cells which causes genes to turn on or off.

Please replace the paragraph beginning at page 10, line 23 with the following amended paragraph:

As used herein, slow turning lateral vessel (STLV)[[÷]] refers to one specific size and shape of a rotating wall vessel.

Please replace the paragraph beginning at page 10, line 25 with the following amended

paragraph:

As used herein, differential display[[÷]] refers to displaying on a filter, gel or chip a discrete set of genes turned on or off in a cell under two different conditions.

Please replace the paragraph beginning at page 10, line 28 with the following amended paragraph:

As used herein, simulated microgravity[[÷]] refers to balance of gravity by oppositely directed forces including shear stresses during rotational wall vessel culture.

Please replace the paragraph beginning at page 10, line 31 with the following amended paragraph:

As used herein, [[]]graded gravitational sedimentation shear[[÷]] refers to the shear imparted to a particle or cell falling through fluid.

Please replace the paragraph beginning at page 10, line 33 with the following amended paragraph:

As used herein, functional protein[[÷]] refers to a protein with biological effects.

Please replace the paragraph beginning at page 10, line 35 with the following amended paragraph:

As used herein, three-dimensional co-culture process[[÷]] refers to cells grown in a matrix or on beads (or other three-dimensional structural support~~suport~~) in a three-dimensional array, rather than on a flat plate.

Please replace the paragraph beginning at page 11, line 1 with the following amended paragraph:

As used herein, Coriolis ~~coriolus~~ force[[÷]] refers to an incidental flow field caused by the rotating gravity vector in the rotating wall vessel.

Please replace the paragraph beginning at page 11, line 3 with the following amended paragraph:

As used herein, shear stress[[÷]] refers to the force felt at the surface of the particle as it moves through the fluid.

Please replace the paragraph beginning at page 11, line 5 with the following amended paragraph:

As used herein, gravity induced sedimentation[[÷]] refers to the force on a particle in the rotating wall vessel making it fall through the fluid due to gravity.

Please replace the paragraph beginning at page 11, line 7 with the following amended paragraph:

As used herein, centrifugal force[[÷]] refers to the force on a particle in the rotating wall vessel which pulls it towards the wall due to rotational speed.

Please replace the paragraph beginning at page 11, line 9 with the following amended paragraph:

As used herein, transcription factor decoy[[÷]] refers to an oligonucleotide folded to form a double stranded DNA which binds a nuclear transcription factor. The transcription factor decoy prevents the transcription factor from binding promoter regions regulating expression of specific genes.

Please replace the paragraph beginning at page 13, line 15 with the following amended

paragraph:

Analysis of the Endosomal Distribution of Megalin and Cubulin by Flow Cytometry

To quantitate the total and endosomal expression of cubulin, megalin, and aquaporin-2 cells in conventional culture, stirred fermentors, and slow turning lateral ~~vessel rotating wall~~ vessels, 0.3 mg/ml 10S fluorescein-dextran was added to each cell culture for 10 minutes at 37[[o]]ºC in the CO_2[[2]] incubator. This step loads an entrapped fluorescent dye into the early endosomal pathway (9, 47). Cells were then immediately diluted into ice cold phosphate buffered saline and washed once. Next, the cells were homogenized with 6 passes of a tight fitting glass-Teflon motor driven homogenizer. A post-nuclear supernatant was formed as the 11,000 g supernatant, 180,000 g pellet of membrane vessels (~~Figure 2A~~ FIGS. 2A-2C).

Please replace the paragraph beginning at page 15, line 12 with the following amended paragraph:

Genetic Decoys

Double stranded genetic decoys matching the sequence of a known shear stress response element were synthesized (Chemicon International Inc., La Jolla, Calif.) (structure and sequence shown ~~at top of Figure 4~~ in FIG. 4A). These decoys had a terminal ~~phosphothiorate~~ phosphothioate moiety to prevent intracellular lysis, and a phosphodiester backbone to facilitate passage across cell membranes (49). Passage to and accumulation in the nuclear compartment of cultured cells was confirmed by confocal imaging of a fluorescein tagged decoy. Three decoys were synthesized: the active decoy, a random sequence control in which the six bases of the shear stress response element were scrambled, and a fluorescein conjugated form of the decoy. Decoys were placed in the cell culture medium of rat renal cortical cells grown as above in conventional two-dimensional culture. Aliquots of cells exposed to control or active sequence decoy at 80 nm concentration were harvested at 2, 6, and 24 hours after exposure.

Please replace the paragraph beginning at page 17, line 15 with the following amended paragraph:

EXAMPLE 14

Culturing ~~Hapatocytes~~ Hepatocytes and Assay for Production of Erythropoietin

As hepatocytes are a source of erythropoietin secreted into the circulation, immortalized human ~~hepatocyes~~ hepatocytes were cultured under control and ~~applied~~ subjected to shear stress conditions. The Hep3B cells were placed into culture in DMEM with 10% fetal bovine serum in a static flask culture. The resultant culture was split, one half remaining in static flask culture and the other half ~~was~~ inoculated into a HARV for culture under increased shear stress conditions. The cells aggregated on the beads. After 24 hours of growing the Hep3B cells in a HARV, erythropoietin was assayed in the cell supernatant. The media were assayed by RIA. The static flask media was also assayed as the control.

Please replace the paragraph beginning at page 18, line 18 with the following amended paragraph:

The ultrastructure of cultures of pure proximal tubular cells or renal cortical cell mixtures of human kidneys were grown in rotating wall vessels for 16 days, and were examined by transmission electron microscopy (FIGS. 1B and 1C). Quantitation of the number of microvilli present by counting random plates at the same magnification demonstrates not only that the rotating wall vessel induces microvillus formation, but co-culture with the normal mix of renal cortical cells increases the effect (Table 1). Normal cortical cell mix in conventional two-dimensional culture has ~~2-1~~ 21 microvilli per field; "pure" proximal tubular culture in rotating wall vessel has ~~10-4~~ 104 microvilli per field; and the normal cortical cell mix[[.]] in rotating wall vessel has ~~35-11~~ 3511 microvilli per field.

Please replace the paragraph beginning at page 26, line 32 with the following amended paragraph:

Study of the mechanisms of action of the rotating wall vessel to induce gene and protein expression during cell culture has been hampered by nomenclature. First, the attachment of the moniker "simulated microgravity", based on engineering analysis of boundary conditions, clouds intuitive analysis of the cell biology as there is no cellular equivalent for this term (1, 6-7). Similarly, the reduced shear stress in the rotating wall vessel compared to stirred fermentors leads to the term "reduced shear stress culture" (1), whereas there is increased shear stress compared to conventional 2-dimensional culture (1, 5). As cell aggregates remain suspended in the rotating wall culture vessels, gravity is balanced by an equal and opposite force. Engineering arguments on the relative contributions of fluid shear, drag, centrifugal force, Coriolis ~~coriolus~~ motion, and tangential gravity-induced sedimentation are themselves tangential to the cell biology. Several lines of evidence are documented to indicate that shear stress responses are one of the ~~component~~ components of the biological response. This research opens the door for analysis of other biological response mediators in the vessels[[,]] and for investigation as to whether unloading of gravity plays as big a role as the oppositely directed balancing forces.